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construct a binary molecular gate which can be controlled by the effector molecule lactose. The device is constructed of three DNA strands which form an elongated hairpin structure of two dsDNA arms with a short ssDNA hinge at one end, and two ssDNA regions on the opposite end. A fourth ssDNA molecule (the 'locking' strand), complementary to the two ssDNA regions, binds by complementary base pairing to seal the gate in the closed configuration. Addition of lac repressor protein to the device causes a displacement of the locking strand by binding to two lac operators embedded in the double-stranded hairpin structure. Binding of the proteins is rapid and sufficiently strong to cause a displacement of the locking strand, switching the gate into the open conformation. Addition of lactose quickly causes a release of the lac repressor protein from the operators allowing the gate to be closed by the binding of the locking strand. The opening and closing of this binary gate was detected by gel shift assays and by fluorescence resonance energy transfer spectroscopy of two dyes located near the opening of this gate. The gate could be cycled repeatedly, responded uniquely to lactose, and may be useful as a device for moving or holding structures on a molecular scale.

2690-Pos Board B709
Nanoparticle Surface Charge Directs the Cellular Binding of Nanoparticle-Protein Complexes
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Nanoparticles are commonly utilized in biological systems as imaging probes, drug delivery agents and in vivo sensors. In most biological applications, the nanoparticle is exposed to a complex mixture of extracellular proteins that adsorb non-specifically to the nanoparticle surface. The resulting ''protein corona'' can dominate the interactions of the nanoparticle with the cellular membrane. We have focused on the role of nanoparticle surface charge in the cellular binding of nanoparticles in the presence of extracellular proteins. Cationic, amine-modified polystyrene nanoparticles and anionic, carboxylate-modified polystyrene nanoparticles were studied as a model system. The cellular binding of cationic and anionic nanoparticles is distinctly different, determined from fluorescence microscopy experiments. For cationic nanoparticles, the cellular binding is increased in the presence of serum proteins. In comparison, anionic nanoparticle binding is inhibited by the presence of serum proteins. Competition assays performed with flow cytometry allowed us to quantify differences in binding and to identify the cellular receptors used by the nanoparticle-protein complexes. We have determined that complexes formed with anionic nanoparticles bind to native protein receptors, while those formed with cationic nanoparticles bind to scavenger receptors. These results indicate that for nanoparticles used in biological applications, the initial surface charge of the nanoparticle mediates cellular binding. Currently, we are extending our studies to nanoparticles used for drug and gene delivery.

Single Molecule Techniques III

2691-Pos Board B710
Quantitative Imaging of Protein Complexes using TIRF Microscopy
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The dynamic composition of protein complexes can have a profound impact on their function. However, conventional pull-down assays cannot provide dynamic information, and sample preparation may affect complex stability. Here, we combine total internal reflection (TIRF) imaging with quantitative image and data analysis to examine the stoichiometry of protein complexes as well as the dynamics of protein associations. Fluorescently labeled proteins are immobilized on glass coverslips, and perfused with other fluorescently tagged protein or small molecules. Complexes are imaged on a TIRF microscope to selectively illuminate bound proteins. We determine copy number and relative positions of fluorescent proteins inside complexes by counting photobleaching vents, using computational super-resolution and mixture-model fitting algorithms for spot detection. We then statistically correct the results for experimental artefacts, such as expression levels and pre-bleaching of fluorophores, which can reach levels of 20%. We further determine binding kinetics by measuring dwell time of fluorescently labeled proteins on immobilized substrates using the same computational approach. Together these methods have allowed us to determine that CENP-A exists in octameric nucleosomes throughout the cell cycle, that myosin adopts multiple conformations on the cortex of the C. elegans zygote that are differentially sensitive to perturbations, and that the interaction of KNL2 with CENP-A in depends on its phosphorylation and its Myb-domain structure.

2692-Pos Board B711
Superresolution Imaging of RNAP and Ribosomes in Live E. Coli
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Superresolution fluorescence imaging is used to locate and track ribosomes, RNA polymerase, and various DNA loci in live E. coli. The strong degree of DNA-ribosome segregation indicates that most protein production occurs on free mRNA transcripts that have diffused into the ribosome-rich regions, not by co-transcriptional translation. The predominant ribosome diffusion coefficient (about 80% of the population) is D_{ribo} = 0.04 μm^2-s^{-1}, attributed to free mRNA being translated by multiple 70S ribosomes (polysomes). A smaller, faster population is attributed to 30S subunits searching for a translation initiation site. New quantitative estimates of ribosome and RNAP copy numbers show that ribosome concentration is more tightly controlled than RNAP. The RNAP diffusion is heterogeneous. About half the population sub-diffuses very slowly (like DNA loci) and half diffuses freely with D_{RNAP} = 0.15 μm^2-s^{-1}. We attribute the slow component to copies that are transcribing or stalled while specifically bound to DNA and the fast component to ''hopping and sliding'' copies. In long RNAP trajectories we observe transitions between slowly and rapidly diffusing states, suggestive of initiation or termination of transcription events. These data provide a more detailed, quantitative picture of how the components of the transcription/translation machinery work together in space and time.

2693-Pos Board B712
Movement on Uneven Surfaces Displays Characteristic Features of Hop Diffusion
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Diffusion in cell plasma membranes is reported to be appreciably slower than diffusion in artificial membranes. We suspect that this is largely an artifact resulting from 2D interpretation of 3D movement over an uneven surface, which leads to the consequent underestimation of the net movement (1). Transient anchorage and/or confinement in domains can easily be explained by topographical trapping. The most serious flaw in the current methods of diffusion measurement is the assumption that the membrane is smooth. When the membrane is uneven, the straight-line-distance between two points requires that the particle leaves the surface, which underestimates the distance travelled. Clearly this is an illegitimate move since both lipids and proteins are restricted to movement within the plane of the membrane. We have therefore developed a new method for analyzing tracks that confines the path to the surface - the shortest within surface distance (SWSD). Simple diffusion was simulated over smooth and uneven surfaces created on a 3D orthogonal grid using an array of connected voxels starting a conditional dilation at the first location that spreads over the surface for a defined number of steps. Notably, after a few steps the diffusion coefficient was higher than after more steps - a characteristic feature of the hop diffusion. We conclude that the minimum requirement for accurate diffusion measurements on the plasma membrane is high resolution topographical images that can be used to assess the effect of local topography on simple diffusion. Only if there is a discrepancy between the measured diffusion coefficient and that expected from Brownian motion within the plane of the membrane after accounting for cell topography is it relevant to invoke anomalous diffusion.
1. Adler et al. (2010) Nat. Methods 7, 170-171

2694-Pos Board B713
Single-Molecule Super-Resolution Microscopy at Video-Rate using Novel SCMOS-Specific Localization Algorithms
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Single molecule localization based super resolution microscopy relies on precise and accurate localization of a large number of single molecules. However, the necessity of accumulating large numbers of localization estimates limits the time resolution to typically seconds to minutes.
Two of the major limitations are the acquisition speed and the photon budget. Replacing EMCCD cameras which are usually implemented in such experiments with recently introduced SCMOS cameras results in a leap in both acquisition speed and effective quantum efficiency. However, the intrinsic pixel-dependent Gaussian noise of the SCMOS cameras introduces localization artifacts and greatly reduces the reliability of the results.
Here, we present a set of specially designed statistics-based algorithms that allows for the first time to fully characterize an SCMOS camera and perform unbiased and precise localization analysis. Using this method we demonstrate